#18 pfarma 1-25-01

Docket No. 47653.2 (1789)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	J.C. Houck, et al.		
Serial Number:	09/190,043	Art Unit:	1631
Filed:	November 10, 1998	Examiner:	M. Borin
For:	SMALL PEPTIDES AND METHODS FOR TREATMENT OF ASTHMA AND INFLAMMATION		

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231 on January 16, 2001.

Deborah Barfield
Deborah Barfield

Honorable Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF JAMES CLAGETT

I, James Clagett, hereby declare that:

1. I am a citizen of the United States of America residing at 5615 139th Avenue SE, Snohomish, Washington 98290 and I am one of the Applicants in the above application.

2. I hold a Ph.D. in microbiology from the University of Nebraska. I have over 30 years experience in research and development related to microbiology, particularly

in the fields of immunology and immunopathology. A copy of my curriculum vitae is attached hereto as Attachment A.

3. Since 1997, I have been a consultant providing scientific expertise to the biotechnology and pharmaceutical communities.

4. I personally performed or directly oversaw the experiments which produced the results presented and discussed herein.

5. I have read and understand the Office Action of August 14, 2000, including the references cited therein.

6. The present invention is directed to method for treatment of allergies using the peptide f-Met-Leu-Phe-Phe, which has among others the properties of inhibiting mast cell degranulation as well as inhibiting neutrophil degranulation and chemotaxis.

7. Based on my knowledge and experience in the field, it is my opinion that, prior to the present invention, it was well known to those skilled in the art that formyl methionyl peptides have pro-inflammatory activity. In addition, it had been suggested by Gleisner et al that f-Met-Leu-Phe can inhibit mast cell degranulation whereas it activates neutrophils and promotes chemotaxis. Indeed, Kermode et al. and Anderson et al. also taught the inflammatory properties of f-met peptides.

8. However, surprisingly, we have discovered that f-Met-Leu-Phe-Phe, can provide a useful anti-inflammatory effect in that it inhibits mast cell degranulation and recruitment of cells to the site of inflammation.

9. In the Office Action dated August 14, 2000, the examiner states:

> . . . the essential difference [is] the effect of a biological mediator (such as F-Met peptide) when it is used alone as compared to its use in the presence of another pro-inflammatory agent. Cellular response to f-Met peptides (which can be described as inflammatory response) is the same type of reaction which mediates response of the organism to a foreign infection. It is well known in the art that biological mediators such as chemotactic factors stimulate the migration of neutrophils from circulation into sites of infection or tissue damage. These mediators are also believed to increase cell adhesion to injured sites and to activate neutrophils to release toxic agents such as oxygen metabolites and proteases. Thus, in the presence of a provoked infection the response caused by f-Met peptides have protective, anti-inflammatory function.

10. It is true that the prior art teaches that:

> Cellular response to f-Met peptides (which can be described as inflammatory response) is the same type of reaction which mediates response of the organism to a foreign infection. It is well known in the art that biological mediators such as chemotactic factors stimulate the migration of neutrophils from circulation into sites of infection or tissue damage. These mediators are also believed to increase cell adhesion to injured sites and to activate neutrophils to release toxic agents such as oxygen metabolites and proteases

However, those responses are "pro-inflammatory" responses. The claimed composition of the present invention blocks those responses. Thus, the claimed composition has an "anti-inflammatory" response.

11. The examiner also states and concludes that:

> Thus Kermode discloses that formyl Met peptides, such as f-Met-Leu-Phe, f-Met-Leu-Phe-Phe and f-Nle-Leu-Phe-Tyr as functional equivalents. In particular, f-Met-Leu-Phe-Phe (i.e., the peptide used in the instant method) is one of the most potent formyl Met peptide analogs.

> ..... Therefore, it would have been *prima facie* obvious to use the peptide f-Met-Leu-Phe-Phe as anti-allergic agent because Gleisner teaches that formyl-Met peptides can reduce anti-histamine release caused by other inflammatory agent (i.e., to cause anti-allergic effect) and Kermode teaches that f-Met-Leu-Phe-Phe is one of the most potent formyl-Met peptides.
>
> ..... Characteristically, . . . the effect of the claimed composition is demonstrated only as inhibitor of inflammatory effect caused by another f-Met peptide, fMLP. The absence . . . of showing of the effect of fMLPP alone is not surprising because Kermode shows (Table 2) that fMLPP (the peptide of the claimed composition) is more potent chemotactic agent and stimulator of neutrophil degranulation than fMLP (the peptide used as "pro-inflammatory" agent). One would expect that fMLPP, alone, would be at least as "pro-inflammatory" as fMLP.

12. However, Kermode conducted *in vitro* tests using rabbit neutrophils that were suspended in solution containing salts, BSA, buffer and for some tests glucose. We have found that such *in vitro* tests are not a predictor of the bioactivity of fMLPP *in vivo*. Although based on the teachings of the prior art, " [o]ne would expect that fMLPP, alone, would be at least as 'pro-inflammatory' as fMLP," as concluded by the examiner, that is an erroneous expectation. Further, based on the teachings of the prior art, one of ordinary skill in the art would not expect fMLPP to act any differently after prior treatment with fMLP.

13. To satisfy the examiner's curiosity about the effect of fMLPP alone, the following experiments have been performed under my direction and control.

Briefly, (i) 200 µg of fMLP alone; (ii) 200 µg fMLPP (HK-X) alone; (iii) 200 µg of fMLP and 200 µg of HK-X administered simultaneously; and (iv) as a control the vehicle of HK-X (4% DMSO in Tyrode's solution) each were injected subcutaneously into the dorsum of the feet of female Balb/CJ female mice. Animals were sacrificed at 30 minutes post-injection and the feet collected for histological examination. The

cutaneous soft tissues were dissected from the bones, fixed in 10% (v/v) neutral buffered saline and embedded in paraffin. Five – seven micron sections were cut and stained with H&E for the detection of cellular content and location within the muscularis and dermis. The numbers of neutrophils in the extravascular zone, occupying 2,200 u^2 around blood vessels, were counted. For each specimen, a total of 10 vessels were counted. The means and SE are presented. Differences between groups were determined by one-way ANOVA followed by the appropriate post hoc tests using SigmaStat version 2.0 software (SPSS Inc., Chicago, IL). The results are illustrated in the figures attached hereto.

14. Figure 1 shows the polymorphonuclear cell count outside the vessels, 30 minutes after injection of 200 µg fMLP alone; 200 µg fMLP and 200 µg HK-X together; 200 µg HK-X alone; and vehicle alone, into the subcutaneous tissues of the dorsum of mice feet.

- Injection of 200 µg of fMLP alone resulted in 24 neutrophils per 2,200 u^2 in the intercellular matrix.
- In specimens from treatment with HK-X alone, results were substantially identical to the control (vehicle treatment alone) ($p<0.05$).
- The simultaneous exposure of fMLP and HK-X resulted in a 91 % **inhibition** of neutrophils diapedesis compared to fMLP alone ($p<0.05$).

15. Figure 2 is a microphotograph comparing stained tissue sections harvested from mice 30 minutes after injection with (i) 200 µg of fMLP alone, (ii) 200 µg fMLP and 200 µg HK-X simultaneously and (iii) 200 µg of HK-X alone into the subcutaneous layers of the skin on the dorsum of mice feet.

- Panel A shows the results of injection of 200 µg of fMLP alone. FMLP treated mouse skin shows the influx of the neutrophils in the surrounding areas of the blood vessel (BV). The neutrophils are located in the connective tissue area (Arrows).
- Panel B shows the results of 200 µg fMLP and 200 µg HK-X when injected simultaneously. The mouse skin shows no cellular infiltration at the surrounding areas of the blood vessel (BV). Occasionally, neutrophils are seen. (Arrows).
- Panel C shows the results of injection of 200 µg of HK-X alone. The treated mouse skin shows no changes in connective tissues and the surrounding area of the blood vessel (BV).

16. Figure 3 shows a higher power examination of specimen similar to those in figure 2.

- Panel A shows the subcutaneous tissue of an HK-X treated mouse. The subcutaneous tissue is normal. The blood vessels (BV) show no neutrophils. Only occasionally a neutrophil is observed (Arrow).
- Panel B is the vehicle control treated mouse. The subcutaneous tissue shows no sign of the effect of a vehicle solution injection.
- Panel C shows the results of 200 µg fMLP and 200 µg HK-X when injected simultaneously. The subcutaneous tissue shows very little change in the histology except for a few neutrophils attached on the endothelial cell surface (Arrow heads). Neutrophils are observed but in very little numbers as compared to the fMLP treated skin tissue (Panel D).

- Panel D shows the results of shows the results of injection of 200 μg fMLP alone. The subcutaneous tissue was heavily infiltrated with neutrophils 30 minutes after injection of fMLP as seen in the surrounding area of the blood vessel (BV). Many neutrophils are also seen attached to the surface of endothelial cells.

17. Based on my knowledge and experience in the art, from these experiments, it can be concluded that fMLP (prior art) administration into the skin of mice produced an intense accumulation of polymorphonuclear cells, largely neutrophils. In contrast, fMLPP (HK-X) has no significant effect when administered alone. However, in connective tissues HK-X administered simultaneously with an equal amount of fMLP totally prevented the accumulation and diapedesis of polymorphonuclear cells into interstitial spaces while administration of HK-X alone showed no inflammatory recruitment of polymorphonuclear cells or other inflammatory cells in the connective tissues. It can also be concluded that HK-X blocks the earliest events triggered by fMLP in the inflammation cascade, that is, recruitment of inflammatory cells.

18. It is my understanding that the Examiner contends that, based on Kermode et al, "one would expect that fMLPP, alone, would be at least as "pro-inflammatory" as fMLP." The above described experiments show the opposite result, that is, fMLPP is anti-inflammatory and also inhibits the pro-inflammatory effects of fMLP.

19. It is also my understanding that the Examiner contends, based on Gleisner, formyl Met peptides are capable of reducing effect of other pro-inflammatory

agents (such as 48/40, anti-rat IgE, etc) in that they inhibit the evoked mast cell degranulation and thus histamine release, but remain a powerful chemotactic agent for mast cells and neutrophils.

20. The above experiments show that f-Met-Leu-Phe-Phe **inhibits** inflammation at the **earliest stages**, i.e., the recruitment of cells to the site of inflammation, so that mast cells are **not** recruited and therefore, Gleisner's teachings have no bearing on the present application. However, f-Met-Leu-Phe ***fails*** to inhibit inflammation.

21. The Examiner refers to the Oxford Dictionary of Biochemistry and Molecular Biology, 1997, p. 43, which states that "antihistamine drugs are used in the treatment of allergy reactions. Based on my knowledge, formyl Met peptides have never been classified as antihistamine drugs and, indeed, had been known to exhibit highly pro-inflammatory activity.

22. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and, further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Codes, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

01/16/2001
Date

James Clagett
James Clagett